Extensive studies on the mechanism and kinetics of the *E. coli* thioredoxin reductase have been conducted and reported. The high sequence similarity between the *E. coli* and *S. aureus* species of thioredoxin reductase suggests that they conduct catalysis in a similar manner. The transfer of electrons occurs from NADPH to the bound FAD cofactor, from the $FADH_2$ cofactor to the Cys 135 - Cys 138 disulfide, and then from the reduced cysteines to the dithiol of thioredoxin (Figure 1). Thioredoxin reductase (which exists as a homodimer) is a two domain protein comprised of a FAD binding domain and a NADPH binding domain. The NADPH binding site includes the active site cysteines, Cys 135 and Cys 138. A third binding surface to which the other substrate thioredoxin must bind has been proposed to be created by a rotation of the NADPH binding domain. To date, only the thioredoxin reductase-FAD-Cys-Cys form of the enzyme from *E. coli* and *Arabidopsis thaliana* have been characterized structurally, although a model for the NADPH bound form of the enzyme has been proposed (Kuriyan et al., *Nature*, 352:172-74 (1991); Waksman et al., *J. Mol. Biol.*, 236:800-16 (1994); and Dai et al, *J. Mol. Biol.*, 264: 1044-57 (1996)). A very recent report describes the X-ray crystal structure of the $FADH_2$ form of the *E. coli* thioredoxin reductase and shows the flavin adopting a 34° butterfly conformation (Lennon et al., *Protein Science*, 8:2366-79 (1999)).

Please replace the paragraph at page 10, lines 12-15, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, page A1, with notations to indicate the changes made.

Figure 4 illustrates anomalous difference Patterson Maps for Patterson section w = 0. Figures 4a and 4b are the Patterson Maps for two different data sets. The peaks in this Patterson map section result from the two fold non-crystallographic symmetry relating the two monomers in the asymmetric unit.

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For: CRYSTALLIZATION AND STRUCTURE DETERMINATION OF *STAPHYLOCOCCUS AUREUS* THIOREDOXIN REDUCTASE

Please replace the paragraph at page 14, line 16 to page 15, line 2, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, page A2, with notations to indicate the changes made.

S. aureus thioredoxin reductase shows the same overall fold and domain structure as observed in the *E. coli* and *A. thaliana* thioredoxin reductases (Kuriyan et al., *Nature*, 352:172-74 (1991); Waksman et al., *J. Mol. Biol.*, 236:800-16 (1994); and Dai et al, *J. Mol. Biol.*, 264: 1044-57 (1996)). The enzyme is composed of two domains classified as the FAD binding domain and a proposed NADPH binding domain (Figure 7). The FAD domain has a central five stranded parallel β sheet surrounded by three α helices on one side and a three stranded antiparallel β sheet on the other. The second domain, the NADPH domain, has a central four stranded parallel β sheet surrounded by two helices on one side and a three stranded antiparallel β sheet on the other. The isoalloxazine ring of the flavin cofactor rests at the interface of these two domains adjacent to the two active site cysteines, Cys135 and Cys 138, which are involved in the redox cycle with the protein substrate thioredoxin. In this crystal structure, the two cysteines form a disulfide bond as indicated by the electron density map (Figure 8). The presence of two active site cysteines will necessitate vigilance to ensure compounds identified by high throughput screening are not acting by electrophilic substitution to these residues.

Please replace the paragraph at page 23, lines 11-24, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, page A2, with notations to indicate the changes made.

For example, a system for reading a data storage medium may include a computer comprising a central processing unit ("CPU"), a working memory

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which may be, e.g., RAM (random access memory) or "core" memory, mass storage memory (such as one or more disk drives or CD-ROM drives), one or more display devices (e.g., cathode-ray tube ("CRT") displays, light emitting diode ("LED") displays, liquid crystal displays ("LCDs"), electroluminescent displays, vacuum fluorescent displays, field emission displays ("FEDs"), plasma displays, projection panels, etc.), one or more user input devices (e.g., keyboards, microphones, mice, touch screens, etc.), one or more input lines, and one or more output lines, all of which are interconnected by a conventional bidirectional system bus. The system may be a stand-alone computer, or may be networked (e.g., through local area networks, wide area networks, intranets, extranets, or the internet) to other systems (e.g., computers, hosts, servers, etc.). The system may also include additional computer controlled devices such as consumer electronics and appliances.

Please replace the paragraph at page 26, line 25 to page 27, line 8, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, page A3, with notations to indicate the changes made.

Thus, this method involves generating a preliminary model of a molecule or molecular complex whose structure coordinates are unknown, by orienting and positioning the relevant portion of *S. aureus* thioredoxin reductase or the *S. aureus* thioredoxin reductase/ligand complex according to Table 1 within the unit cell of the crystal of the unknown molecule or molecular complex so as best to account for the observed x-ray diffraction pattern of the crystal of the molecule or molecular complex whose structure is unknown. Phases can then be calculated from this model and combined with the observed x-ray diffraction pattern amplitudes to generate an electron density map of the structure whose coordinates are

unknown. This, in turn, can be subjected to any well-known model

building and structure refinement techniques to provide a final, accurate

structure of the unknown crystallized molecule or molecular complex (E.

Lattman, "Use of the Rotation and Translation Functions," in Meth.

Enzymol., 115, pp. 55-77 (1985); M.G. Rossman, ed., "The Molecular

Replacement Method - A Collection of Papers on the Use of Non-

Crystallographic Symmetry," Int. Sci. Rev. Ser., No. 13, Gordon &

Breach, New York (1972)).

Please replace the paragraph at page 33, lines 30 to page 34, line 7, with the following

rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, page A4,

with notations to indicate the changes made.

    Useful programs to aid one of skill in the art in connecting the individual

chemical entities or fragments include, without limitation, CAVEAT (P.A.

Bartlett et al., in "Molecular Recognition: Chemical and Biological Problems,"

Special Publ., Royal Chem. Soc., 78:182-196 (1989); G. Lauri et al., *J. Comput.

Aided Mol. Des.*, 8:51-66 (1994); available from the University of California,

Berkeley, CA); 3D database systems such as ISIS (available from MDL

Information Systems, San Leandro, CA; reviewed in Y.C. Martin, *J. Med. Chem.*,

35:2145-2154 (1992)); and HOOK (M.B. Eisen et al., Proteins: Struc., Funct.,

Genet. 19:199-221 (1994); available from Molecular Simulations, San Diego,

CA).

Please replace the paragraph at page 34, lines 8-16, with the following rewritten

paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, page A4, with

notations to indicate the changes made.

PRELIMINARY AMENDMENT
Applicant(s):   Timothy E. Benson
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Confirmation No.: 2707
Filed: April 3, 2001
For: CRYSTALLIZATION AND STRUCTURE DETERMINATION OF *STAPHYLOCOCCUS AUREUS* THIOREDOXIN REDUCTASE

S. aureus thioredoxin reductase binding compounds may be designed "de novo" using either an empty binding site or optionally including some portion(s) of a known inhibitor(s). There are many de novo ligand design methods including, without limitation, LUDI (H.-J. Böhm, *J. Comp. Aid. Molec. Design*, 6:61-78 (1992); available from Molecular Simulations Inc., San Diego, CA); LEGEND (Y. Nishibata et al., *Tetrahedron*, 47:8985 (1991); available from Molecular Simulations Inc., San Diego, CA); LeapFrog (available from Tripos Associates, St. Louis, MO); and SPROUT (V. Gillet et al., *J. Comput. Aided Mol. Design*, 7:127-153 (1993); available from the University of Leeds, UK).

Please replace the paragraph at page 43, line 18 to page 44, line 9, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, page A4, with notations to indicate the changes made.

The selenomethionine structure solution required the identification of the positions of 14 selenium atoms. There are seven methionines per monomer excluding the N-terminal Met which is usually disordered. Anomalous difference Patterson maps revealed 8 selenium sites whose locations were readily derived using the automated Patterson search algorithm in SHELX (Sheldrick et al., *Acta Cryst.*, B51:423-31 (1995)). Four of the eight sites were able to identify the other four sites using anomalous difference Fourier methods and *vice versa*. Three additional sites were identified in each of the anomalous difference Fourier maps phased with each of the two sets of four sites found by Patterson methods. These three new sites were used for phasing and able to successfully identify the initial eight sites. The eleven sites were analyzed using a graphical display. A definitive two-fold axis was observed and suggested one additional site which did not have a symmetry mate. The last two sites were identified by phasing with the

eleven sites and choosing peaks that should be related by non-crystallographic symmetry. One peak found via this method was a true methionine position, and non-crystallographic symmetry was used to identify the final site. Phasing statistics (Tables 8 and 9) and electron density maps (Figures 5 and 6) indicate that the multiple anomalous dispersion experiment was successful in producing suitable phases of high enough quality to permit a straightforward tracing of the chain. The structure was built in CHAIN (Sack, *Journal of Molecular Graphics*, 6:224-25 (1988)) using the *E. coli* thioredoxin reductase as a starting model and refined using X-PLOR (Brünger, X-PLOR version 3.1, Yale University Press (1992)) to an R-factor of 25.8% and a Free R-factor of 29.4%.

Please replace the paragraph at page 46, line 32 to page 47, line 6, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, page A5, with notations to indicate the changes made.

All refinement cycles were carried out with XPLOR98 (Brünger, X-PLOR version 3.1, Yale University Press (1992)) incorporating bulk solvent correction during the refinement (Jiang et al., *J. Mol. Biol.*, 243:100-15 (1994)). Progress of the refinement was monitored by a decrease in both the R-factor and Free R-factor. Stereochemistry of the model was checked using PROCHECK (Laskowski et al., *J. Appl. Cryst.*, 26:283-91 (1993)) revealing no residues in disallowed regions of the Ramachandran plot. Figures 5-6 and 8 were made using SETOR (Evans, *J. Mol. Graphics*, 11:134-38 (1993)) and Figure 7 was produced with both MOLSCRIPT (Kraulis, *J. Appl. Cryst.*, 24:946-50 (1991)) and Raster 3D (Merritt 1994) while Figures 10, 12-14, and 16-17 were produced in MOLSCRIPT (Kraulis, *J. Appl. Cryst.*, 24:946-50 (1991)) alone.